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The Effect of Cold Acclimation on Gene Expression in the Crayfish, Procambarus clarcii

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The effect of cold acclimation on gene expression in the crayfish, *Procambarus clarkii*

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From *The Crayfish*, by T. H. Huxley, 1879

Abstract

Homeostasis of intracellular calcium is necessary for eukaryotic organisms because Ca^{2+} acts as a second messenger in cells (1). *Procambarus clarkii*, the freshwater crayfish, is considered to be an ideal model for studying Ca^{2+} homeostasis because of its external calcified cuticle and periodic molt cycle (2). Exposure to low-ambient temperature has been shown to cause influx of Ca^{2+} into the cells (3). We studied the expression of sarcoplasmic calcium-binding protein (SCP) and eukaryotic elongation factor 1Bγ (eEF1Bγ) in response to cold-acclimation, in an effort to better understand the role of these proteins in the crayfish. Most notably, SCP expression showed large decreases in expression in both the gill and heart after 7 days of acclimation. eEF1Bγ shows large increases in expression in the gill at day 7, while all other tissues display modest decreases. Day 21 data do not mirror the results seen in day 7, and the reason for this remains undetermined. Hemolymph ion levels were analyzed by cation chromatography after cold acclimation. Magnesium concentration was increased at both 7 and 21 days, while calcium and potassium levels were unchanged.

Introduction

The crayfish molt cycle, during which calcium is moved unilaterally, is a good model system for the study of calcium handling proteins. Cold acclimation causes cellular Ca^{2+} influx, and thus is another means of studying these proteins. We hypothesize that changes in gene expression during the cold response are similar to changes in the molt cycle.

Sarcoplasmic calcium-binding protein (SCP) binds calcium and magnesium (4). It is abundantly expressed in muscle, but can also be found in other crayfish tissue. SCP is downregulated during pre- and postmolt, particularly in epithelial tissue (5).

Elongation factors regulate mRNA translation into polypeptides. Eukaryotic elongation factor 1-B (eEF1B), recruits tRNA to the ribosome. The γ subunit is thought to aid assembly of the eEF1B complex (6).

SCP and eEF1Bγ both decrease during the molt cycle in epithelial tissues (5). Thus, we hypothesized that SCP and eEF1Bγ expression decrease with cold acclimation, especially in epithelial tissues.

Results

SCP and eEF1Bγ expression in day 7 cold-acclimated crayfish

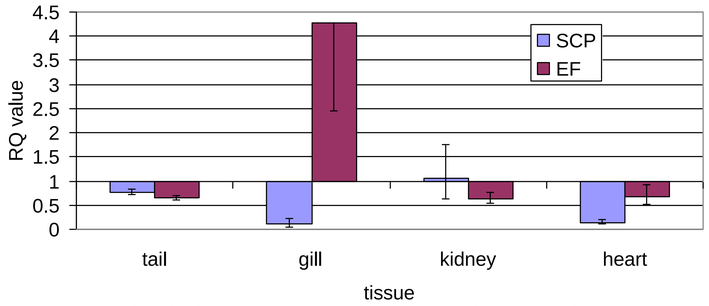

Fig. 2. Expression levels determined with relative quantification real time PCR. N=6 for each tissue type. The calibrator for each tissue is room temperature crayfish, with an RQ value of 1. Error bars are the standard error. SCP decreases dramatically in gill (RQ = .1) and heart (RQ=.15). eEF1Bγ shows a large increase in gill (RQ=4.28).

SCP and eEF1Bγ expression in day 21 cold-acclimated crayfish

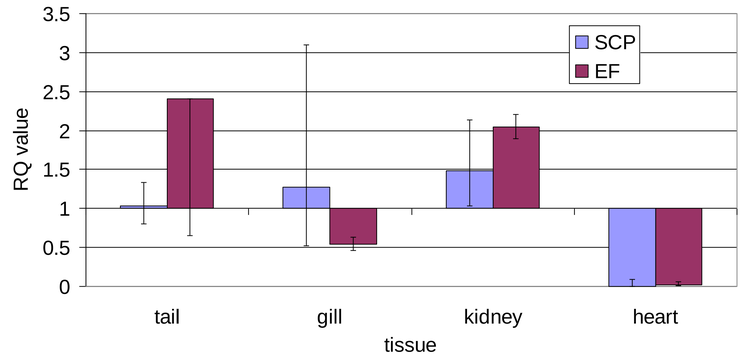

Fig. 3. Expression levels determined with relative quantification real time PCR. N=3 for each tissue type. The calibrator for each tissue is room temperature crayfish, with an RQ value of 1. Error bars are the standard error. SCP shows a dramatic decrease in heart (RQ=.004). EF shows large changes in tail (RQ=2.4), heart (RQ=.02), and kidney (RQ=2.04).

Results

Hemolymph Mg^{2+} concentration with cold acclimation

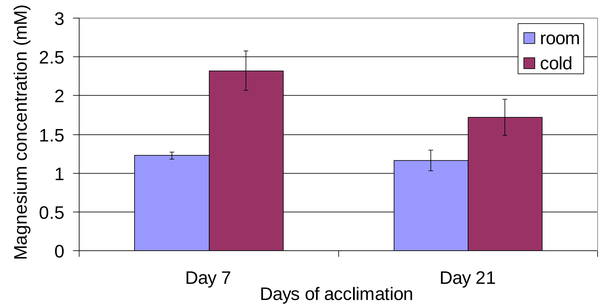

Fig. 4. Concentrations were determined via cation chromatography and PeakNet software. Sample sizes are as follows: room day 7, N=4; cold day 7, N=6; room day 21, N=4; cold day 21, N=3. Error bars are the standard error. K^+ and Ca^{2+} concentrations did not change with cold acclimation.

Summary

- Heart SCP expression decreased after both 7 and 21 days of cold acclimation.
- Gill SCP expression decreased in 7 day acclimated crayfish.
- Tail and kidney showed small and inconsistent changes in SCP expression with cold acclimation.
- eEF1Bγ expression does not show consistent changes with cold acclimation.
- Hemolymph Mg^{2+} levels increased with cold acclimation after both 7 and 21 days, while K^+ and Ca^{2+} remained unchanged.
- The possible connections between decreased heart SCP and increased hemolymph Mg^{2+} are a target for further studies.
- Overall responses of SCP and eEF1Bγ to the cold do not mirror those seen during the molt cycle.

Methods

Crayfish were obtained from Niles, Biological, CA. Care of the animals included feeding of shrimp pellets every third day, cleaning water filters daily, and changing water as necessary. Crayfish were randomly sorted into room temperature or cold (4°C) water. Cold acclimation was conducted for 7 and 21 days. After the desired length of acclimation, tissue samples were collected. This process involved drying and weighing the crayfish, followed by hemolymph sampling, decerebration and dissection. Hemolymph was taken from the joint of the large pincher leg, if possible, or from a walking leg if the pincher had been lost. During tissue removal, the antennal gland (kidney), hepatopancreas (liver), heart, gills, and tail muscle were collected. Tissues were immediately frozen in liquid nitrogen. After tissue collection was completed, all samples were frozen in the -80°C freezer until further use. Hemolymph was diluted (10µL/1000µL deionized water) and frozen until analyzed.

Following collection, each tissue was treated with Stat-60 (Tel-Test, Inc., Friendswood, TX) to isolate the RNA. RNA was cleaned with Turbo DNA-*free* (Ambion, Austin, TX) and cDNA synthesized via TaqMan RT-PCR (Applied Biosystems, Foster City, CA). cDNA was then used to perform real-time PCR, using a SYBR Green PCR kit (Applied Biosystems). 18s rRNA was used as an endogenous control for the reaction, with the following primers: 5'-TGGTGCATGGCCGTTCTTA-3' (sense) and 5'-AATTGCTGGAGATCCGTCGAC-3' (antisense). SCP primers were as follows: 5'-CATTGATGTGAACGGTGACGG-3' (sense) and 5'-AGGCATCGTCGATCTCCTTGA-3' (antisense). The reaction mixture was 20 µL total and contained: 2 µL of 10X SYBR Green PCR buffer, 3 µL of 25 mmol L^{-1} $MgCl_2$, 2 µL of dNTP mix (2.5 mmol L^{-1} dATP, 2.5 mmol L^{-1} dCTP, 2.5 mmol L^{-1} dGTP, and 5 mmol L^{-1} dUTP), .125 µL AmpliTaq Gold (5 U μL^{-1}), .25 µL AmpErase UNG 91 U μL^{-1}, 2 µL template cDNA, and 4.08 µL of each 5 µmol L^{-1} primer in water. 18s rRNA mix used a diluted primer (4.08 µL per 1 µmol L^{-1}) and 1:10 diluted cDNA. PCR reactions were performed in a 96-well plate using the relative quantification ΔΔ Ct method. The Ct (threshold cycle) indicates the PCR cycle at which increase in SYBR Green fluorescence is detected above a baseline signal. PCR conditions were the following: 50°C for 2 min, 95°C for 10 min, and 40 cycles of 95°C for 15 sec followed by 60°C for 1 min. In this way, levels of SCP and EF-1γ mRNA were assessed. Cold-acclimated expression levels were calibrated to room temperature levels to determine relative expression.

Diluted hemolymph samples were analyzed using cation chromatography. We used a Dionex 500-DX chromatography system with a CS12a cation-exchange column, AS50 autosampler with 25-µL injection loop, CSRS-Ultra 4mm recyclable suppressor, and the PeakNet suoftware package. Methylsulfonic acid (18 mmol L^{-1}) was used as an eluent. Each sample was run for 15 minutes at a flow rate of 1 mL min^{-1}. The PeakNet software was used for peak and baseline determination.

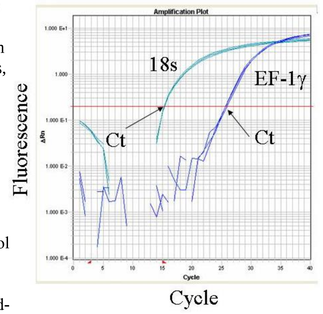

Fig. 1 Representative trace of 18s and eEF1Bγ real-time amplification. Ct indicates the threshold cycle.

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References Cited

[1] Gao, Y., D.R. Whalen, F.M. Vigo, C.M. Gillen, A.E. Golshani, M.G. Wheatly. (2005) Effect of cold acclimation on expression of Ca^{2+} exporting proteins in the freshwater crayfish *Procambarus clarkii*. Submitted to *Journal of Experimental Biology*.

[2] Gao, Y. and M.G. Wheatly. (2004) Characterization and expression of plasma membrane Ca^{2+} ATPase (PMCA3) in the crayfish *Procambarus clarkii* antennal gland during molting. *J. Exp. Biol.* **207:** 2991-3002.

[3] Sangwan, V., B.L. Orvar, J. Beyerly, H. Hirt, R.S. Dhindsa. (2002) Opposite changes in membrane fluidity mimic cold and heat stress activiation of distinct plant MAP kinase pathway. *Plant J.* **31**: 629-638.

[4] Hermann, A. and J.A. Cox. (2005) Sarcoplasmic calcium-binding protein. *Comp. Biochem. Physiol.* **111B:** 337-345.

[5] Gao, Y., C.M. Gillen, M.G. Wheatly. (2006) Molecular characterization of the Sarcoplasmic Calcium-binding Protein (SCP) from crayfish *Procambarus clarkii*. *Comp. Biochem. Physiol.* **144B**: 478-487.

[6] Browne, G.J. and C.G. Proud. (2002) Regulation of peptide-chain elongation in mammalian cells. *Eur. J. Biochem.* **269** 5360-5368.